

Induction of passive immunity in broiler chickens against *Eimeria acervulina* by hyperimmune egg yolk immunoglobulin Y¹

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ABSTRACT The protective effect of hyperimmune IgY fraction of egg yolk prepared from hens hyperimmunized with multiple species of *Eimeria* oocysts on experimental coccidiosis was evaluated in young broilers. Chickens were continuously fed from hatch with a standard diet containing hyperimmune IgY egg yolk powder or a nonsupplemented control diet and orally challenged at d 7 posthatch with 5.0×10^3 sporulated *Eimeria acervulina* oocysts. Body weight gain between d 0 and 10 and fecal oocyst shedding between d 5 and 10 postinfection were determined as parameters of protective immunity. Chickens given 10 or 20% hyperimmune IgY egg yolk powder showed significantly increased BW gain and reduced fecal oocyst shedding compared with control birds fed the nonsupplemented diet. In another

trial, lower IgY concentrations (0.01, 0.02, and 0.05%) were used to treat birds with 1.0×10^4 oocysts of *E. acervulina*. Total oocyst shedding was significantly ($P < 0.05$) reduced in chickens fed the 0.02 and 0.05% hyperimmune IgY supplemented-diets compared with animals fed the nonsupplemented diet. Similarly, chickens fed 0.5% of hyperimmune IgY egg yolk powder diet and challenged with 1.0×10^4 oocysts exhibited reduced oocyst shedding compared with the control birds given 0.5% of IgY from nonimmunized hen eggs, although BW gain was not affected. We conclude that passive immunization of chickens with anti-coccidia IgY antibodies provide protective immunity against coccidiosis challenge infection.

Key words: egg yolk, immunoglobulin Y, coccidiosis, *Eimeria*, passive immunization

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INTRODUCTION

Avian coccidiosis is an intestinal disease caused by several distinct species of *Eimeria* protozoa and is the most economically significant parasitic infection of the poultry industry worldwide (Lillehoj and Lillehoj, 2000). Although prophylactic use of anti-coccidia feed additives has been the primary method of controlling avian coccidiosis, alternative control methods are needed due to increasing concerns with drug use and high cost of vaccines (Lillehoj and Lee, 2007a,b). Therefore, much interest has been devoted toward the development of drug-independent control strategies against coccidiosis (Lillehoj et al., 2005; Lillehoj and Lee, 2007a,b). As an example, our laboratory recently documented enhanced

intestinal immunity and increased resistance to coccidiosis in poultry by dietary feeding of plant-derived phytonutrients and probiotics (Lee et al., 2007a,c, 2008).

An alternative control strategy potentially applicable to intestinal diseases such as avian coccidiosis involves passive immunization using hyperimmune, parasite-specific antibodies. As opposed to pathogen-specific immunity achieved by active vaccination with live or inactivated microorganisms, or subunits derived from pathogens, passive immunization relies on the transfer of humoral immunity in the form of active antibodies from one individual to another (Rosenow et al., 1997). Although polyclonal antibodies from mammals such as rabbits and goats have been commonly used for passive immunization in the past, rising concerns over animal welfare issues are prompting the pharmaceutical industry to explore less invasive alternatives for producing therapeutic antibodies. In this regard, chicken egg yolk IgY antibodies offer a practicable alternative to mammalian serum antibodies because of their feasibility for large-scale commercial production and the relatively noninvasive methods used for their preparation

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(Yokoyama et al., 1992, 1998; Schade et al., 2005). Passive immunity against *Escherichia coli* and *Salmonella* Enteritidis infections using IgY antibodies has been demonstrated in pigs, mice, and cows (Ikemori et al., 1992; Yokoyama et al., 1992, 1998; Zhen et al., 2008).

Previous studies have established a role for passive immunity in avian coccidiosis. Passive transfer of maternal antibodies from hens infected with *Eimeria maxima* to eggs partially protected the young offspring against *Eimeria tenella* challenge infection (Smith et al., 1994). Karim et al. (1996) reported reduced fecal oocyst output following experimental infection with *E. tenella* or *E. maxima* in chickens that were intravenously pretreated with a mouse monoclonal IgM antibody against a major protein of the *E. tenella* oocyst wall. On the basis of these findings, we hypothesized that passive immunization with egg yolk containing hyperimmune anti-coccidia IgY antibodies would confer protection against experimental coccidiosis. To test this hypothesis, a pilot study was carried out to evaluate commercially available hyperimmune egg yolk IgY antibodies that were produced from broiler hens hyperimmunized with multiple *Eimeria* species oocysts and tested its utility as a feed supplement in conferring protective immunity against subsequent challenge infection with *Eimeria acervulina*.

MATERIALS AND METHODS

Preparation of Egg Yolk IgY Antibodies

A commercially available egg yolk powder containing hyperimmune IgY antibodies (Supracox, **SC**; Investigación Aplicada, Sociedad Anónima de Capital Variable, Puebla, Mexico) and a control egg yolk powder were used in this study. Control egg powder was obtained from un-immunized hens. The SC was prepared from egg yolks of specific-pathogen-free broiler hens hyperimmunized with live oocysts of 3 major *Eimeria* species, *E. tenella*, *E. acervulina*, and *E. maxima*. Hyperimmunization of hens was carried out by orally infecting hens with 4,000 sporulated oocysts of *E. tenella*, *E. acervulina*, and *E. maxima*. Immunization of hens started at 60 d of age and continued through the egg production period. The IgY antibodies were obtained by removing the lipid and fatty components with solvents, followed by protein precipitation, and protein purification (Avid AL, Unisyn Technologies, Tustin, CA), and then spray-dried (Yokoyama et al., 1993). The IgY was identified using horseradish peroxidase conjugated goat anti-IgY antibody in ELISA (Pierce Biotechnology, Rockford, IL; Sommerville et al., 2005).

Induction of Passive Immunity and Evaluation of Protective Immunity

Three independent in vivo trials were carried out to evaluate protective immunity against experimental

avian coccidiosis by the measurements of BW gain and fecal oocyst shedding as described (Gabriel et al., 2006; Lee et al., 2008). All experiments were performed following approval by the Beltsville Agriculture Research Center Small Animal Care and Use Committee.

Trial 1. Fifty 1-d-old broilers (Ross/Ross, Longenecker's Hatchery, Elizabethtown, PA) were randomly assigned to 5 groups (10 birds/group) in electrically heated battery units. Two groups (uninfected and infected controls) were fed a normal standard diet, and 3 groups were fed the standard diet supplemented with 5 (**SC5**), 10 (**SC10**), or 20% (vol/vol; **SC20**) SC. At 7 d posthatch, all groups except the uninfected control group were orally challenged with 5.0×10^3 sporulated *E. acervulina* oocysts. Body weights were measured at 0 (preinfection weight) and 10 d postinfection. For fecal oocyst enumeration, birds from the 4 infection groups were placed in collection cages (2 birds/cage) and fecal droppings were collected between 5 and 10 d postinfection. Each fecal material was suspended in 3 L of water, and oocyst numbers were determined in two 35-mL samples using a McMaster chamber according to the following formula: total oocysts/bird = oocyst count \times dilution factor \times (fecal sample volume/counting chamber volume)/2.

Trial 2. Fifty 1-d-old broilers were randomly assigned to 5 groups (10 birds/group). Two groups (uninfected and infected controls) were fed a normal standard diet, and 3 groups were fed the same diet supplemented with 0.01% (SC 0.01), 0.02% (SC 0.02), or 0.05% (SC 0.05; vol/vol) SC. The concentrations were based on the dietary ratios in the previous studies that showed a protective effect against coccidiosis (Lee et al., 2007a,c). The birds were orally challenged with 1.0×10^4 oocysts. Body weight gains and oocyst numbers were measured from 5 groups of birds (10 birds/group), as in trial 1.

Trial 3. Thirty 1-d-old broilers were randomly assigned to 3 groups (10 birds/group). The birds were also orally challenged with 1.0×10^4 oocysts as in trial 2. Body weight gains and oocyst numbers were measured as in trial 2, except uninfected and infected control groups were fed a diet supplemented with 0.5% (vol/vol) of control IgY from nonimmunized hen eggs and the last group was fed a diet supplemented with 0.5% (SC0.5; vol/vol) SC. The supplementary concentration of diet was considered with the previous studies that showed immunostimulating effects in broilers (Lee et al., 2007b, 2008).

Statistical Analysis

Data analyses were performed using SPSS software (SPSS 15.0 K for Windows, Chicago, IL). All data were expressed as means \pm SEM values. Comparisons of the mean values were performed by 1-way ANOVA, followed by the multiple Duncan test ($P < 0.05$). Difference of oocysts between control and SC0.5 in trial 3 was examined using a *t*-test. A $P \leq 0.05$ was considered statistically significant.

RESULTS

Three experimental trials were carried out to assess the effectiveness of feeding hyperimmune egg yolk IgY (SC) as a dietary supplement in conferring passive immunity against *E. acervulina* challenge infection.

Trial 1

As shown in Figure 1A, the birds in the SC10 and SC20 groups exhibited significantly increased BW gains compared with the group fed only the standard diet ($P < 0.05$) following challenge infection with 5.0×10^3 sporulated *E. acervulina* oocysts. Body weight gains in the SC-fed groups were statistically equivalent to that of noninfected animals. Furthermore, the SC10 and SC20 groups displayed significantly reduced fecal oocysts shedding ($P < 0.05$) compared with the *E. acervulina*-infected group that was on the standard diet (Figure 1B).

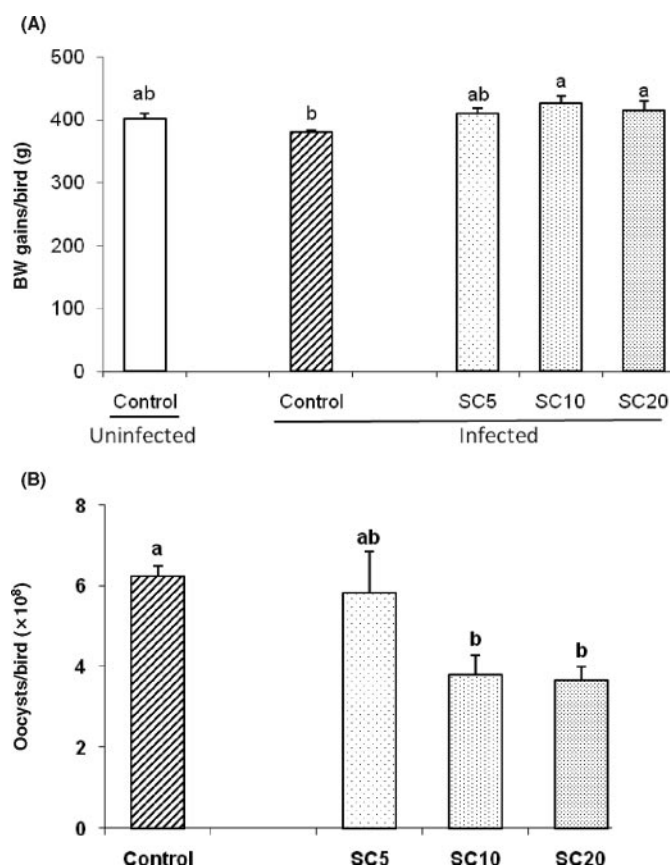


Figure 1. The effect of high doses of Supracox (SC; Investigación Aplicada, Sociedad Anónima de Capital Variable, Puebla, Mexico) and low coccidia challenges on protective immunity to *Eimeria acervulina*. One-day-old broiler chickens were fed a standard diet supplemented with SC at 0% (control), 5% (SC5), 10% (SC10), or 20% (SC20; vol/vol); the chickens were then challenged with 5.0×10^3 sporulated oocysts of *E. acervulina* at 7 d posthatch. Body weight gains between 0 and 10 d postinfection (dpi; A) and fecal oocyst shedding between 5 and 10 dpi (B) were measured. Each bar represents the mean \pm SEM values. ^{a,b}Within each graph, bars not sharing letters are significantly different ($P < 0.05$), according to the Duncan's multiple range test.

Trial 2

The second in vivo trial was carried out using lower doses of SC supplement and a greater dose *E. acervulina* challenge infection. Three groups of broiler birds were fed standard diets supplemented with 0.01, 0.02, and 0.05% SC and challenged with 1.0×10^4 *E. acervulina* oocysts. Although there was a general trend toward increasing weight gains with increasing doses of SC used in this feeding trial, there was no significant difference between the groups fed supplemented diet and standard diet (Figure 2A). However, there was a significant difference in fecal oocyst shedding; the SC0.02 and SC0.05 groups showed significantly reduced fecal oocysts compared with the birds on the standard diet ($P < 0.05$; Figure 2B).

Trial 3

As shown in Figure 3A, there was no significant difference in the BW gain of the infected birds fed SC0.5 and control diets. However, the SC0.5 group displayed significantly reduced fecal oocyst shedding compared with the infected control group given the nonimmune IgY diet ($P < 0.05$; Figure 3B).

DISCUSSION

In this study, the protective effect of oral IgY from eggs of hens hyperimmunized with mixed *Eimeria* oocysts was evaluated on experimental coccidiosis. At the highest dose of SC and using 5.0×10^3 sporulated *E. acervulina* oocysts as a challenge dose, the SC-supplemented diet significantly increased BW gain and reduced fecal oocyst shedding compared with a standard diet. At lower doses of SC and with 1.0×10^4 sporulated *E. acervulina* challenge inoculum, there was no significant effect of SC supplemented diet on BW, but its beneficial effect on reducing fecal oocyst shedding was still significant. However, congruent with the latter observations, the chickens that were fed a SC-supplemented diet demonstrated reduced parasite shedding compared with the birds given nonimmune IgY, similarly prepared from egg yolk. This finding clearly indicates that it is the immunoglobulin portion of SC that is providing protective effects against coccidiosis challenge infection.

Whereas weight gain and oocyst shedding are generally considered as accurate parameters of protective immunity during experimental coccidiosis, it is also appreciated that a lack of correlation between these interrelated variables may occur (Lee et al., 2007a,c). Nevertheless, oral feeding of young birds with diets that have been supplemented with SC reduced fecal oocyst shedding at the dose ranges that we employed in this study. Feeding egg yolk powder that was prepared from un-immunized hens did not show any protective effects on the basis of fecal oocyst shedding and BW gain. The challenge doses of *E. acervulina* oocysts that were

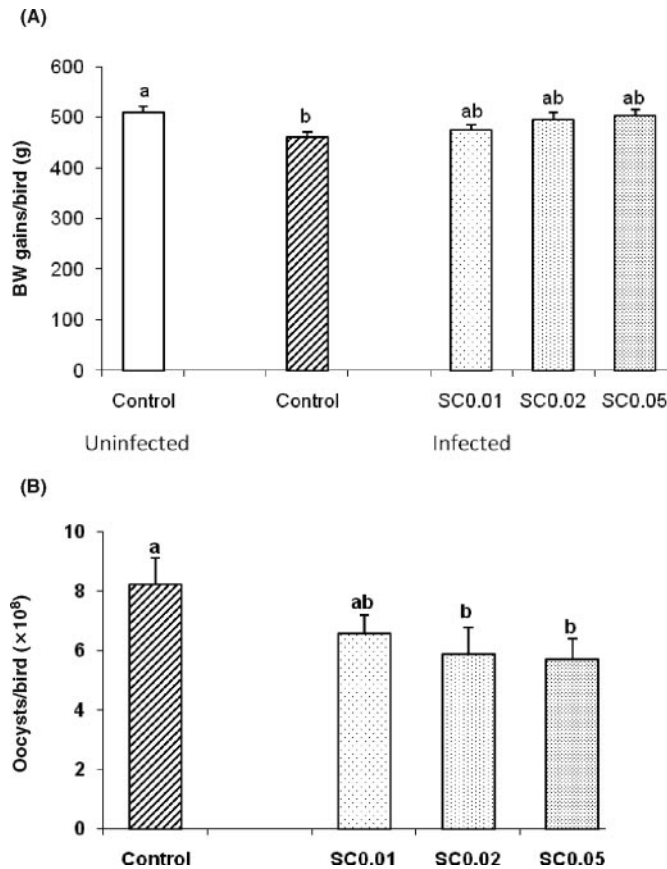


Figure 2. The effect of low doses of Supracox (SC; Investigación Aplicada, Sociedad Anónima de Capital Variable, Puebla, Mexico) and high coccidia challenges on protective immunity to *Eimeria acervulina*. One-day-old broiler chickens were fed a standard diet supplemented with SC at 0% (control), 0.01% (SC0.01), 0.02% (SC0.02), or 0.05% (SC0.05; vol/vol). The chickens were challenged with 1.0×10^4 sporulated oocysts of *E. acervulina* at 7 d posthatch. Body weight gains between 0 and 10 d postinfection (dpi; A) and fecal oocyst shedding between 5 and 10 dpi (B) were measured. Each bar represents the mean \pm SEM values. ^{a,b}Within each graph, bars not sharing letters are significantly different ($P < 0.05$), according to the Duncan's multiple range test.

used in this study may be considerably greater than the levels that commercial birds are likely to be exposed to in some production facilities (Wallach et al., 1995). Therefore, low oral doses of SC may be used to protect coccidiosis in the poultry raised under normal field conditions. Additional studies are ongoing to evaluate the effects of SC against higher oocyst numbers and other *Eimeria* species to extend the current results to the control of coccidiosis in poultry as well as in other food production animals. In addition, mechanistic studies are required to determine how passively administered antibodies bestow protection against coccidia challenge infection, particularly in the context of cell-mediated immunity, which plays the dominant role against avian coccidiosis (Lee et al., 2008). Finally, identification of potential vaccine target proteins of *Eimeria* using protective hyperimmune IgY antibodies will facilitate the development of novel subunit coccidiosis vaccines.

Belli et al. (2004) identified 2 recombinant proteins of the genes, gam56 and gam82, that encode the im-

munodominant components of a commercial subunit vaccine (CoxAbic; not available in the United States) derived from *E. maxima* gametocytes. This vaccine has been shown to induce partial protection against *E. acervulina*, *E. maxima*, and *E. tenella* (Wallach, 2002), but its production is laborious and costly (Belli et al., 2004). Following multiple immunizations with the recombinant proteins, alone or in combination, hens elicited a dose-dependent antibody response indicative of similar antigenic and immunogenic properties to the native protein vaccine. However, the strategy that was used to produce hyperimmune egg yolk IgY antibodies to induce passive immunity against coccidiosis is different from the method using CoxAbic in many aspects.

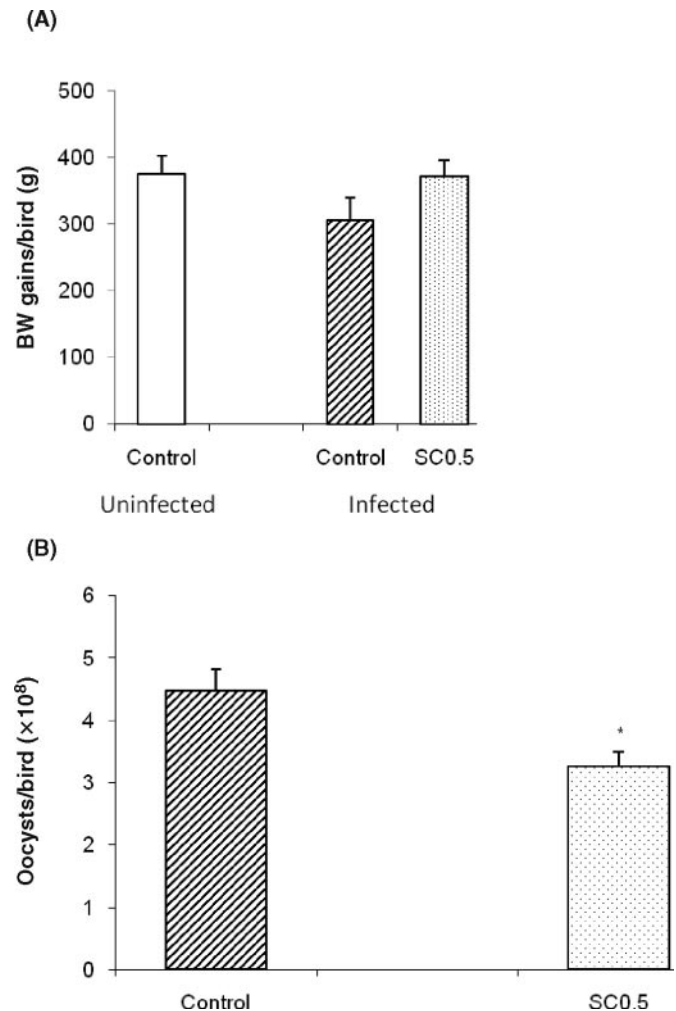


Figure 3. The effect of Supracox (SC; Investigación Aplicada, Sociedad Anónima de Capital Variable, Puebla, Mexico) compared with nonimmune egg yolk IgY on protective immunity to *Eimeria acervulina*. One-day-old broiler chickens were fed a standard diet supplemented with 0.5% (vol/vol) nonimmune IgY (control) or 0.5% SC, the chickens were challenged with 1.0×10^4 sporulated oocysts of *E. acervulina* at 7 d posthatch. Body weight gains between 0 and 10 d postinfection (dpi; A) and fecal oocyst shedding between 5 and 10 dpi (B) were measured. Each bar represents the mean \pm SEM values. Within each graph of BW gain, bars not sharing the indicated letters are significantly different ($P < 0.05$), according to the Duncan's multiple range test. Difference of oocysts between the infected control and the SC0.5 groups was tested by a Student's *t*-test. * $P \leq 0.05$ was considered statistically significant.

First, SC was obtained from hens that have been hyperimmunized with multiple species of *Eimeria* oocysts instead of recombinant proteins. Second, protection induced by SC was dependent upon feeding hyperimmune egg yolk IgY antibodies to young broilers instead of maternally transmitted antibodies. Lastly, passive immunity was elicited as long as birds were fed with SC, whereas protection induced by maternally derived antibodies wanes with time and disappear within 3 wk.

In conclusion, this study demonstrated beneficial effect of using an immune enhancing supplement like hyperimmune IgY antibodies to passively provide significant protection against avian coccidiosis in newly hatched birds. Further studies to improve the effectiveness of hyperimmune IgY in the diet using a better delivery system will be necessary to further enhance the immune enhancing effects of hyperimmune IgY product. Compared with other methods of active vaccination by live parasites, attenuated parasites, or recombinant subunit vaccines, the advantages of the passive immunization strategy are obvious. These include (a) the relative ease and noninvasive method of use in commercial settings, (b) the comparatively low cost of production in utilizing current egg-laying and yolk preparation technologies, and (c) the ability to quickly target unique antigenic variants of *Eimeria* species emerging in particular geographic locales.

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